lights at other drivers, slowing down in front of drivers who are tailgating, and cutting off other drivers). Most participants reported engaging in more passive behaviors, such as shaking their heads at other drivers.

Statistics

FINDING AN OPTIMAL DESIGN USING PSEUDOFACTORS. Mary A. Marion, Department of Statistics, Virginia Polytechnic Institute and State University. This paper was written as a result of an Industrial Systems Engineering project performed at Virginia Tech. This paper reflects an evolving procedure to design an industrial experiment utilizing optimality criteria, AIC statistic and the usual regression/ANOVA model statistics. Discrete factor settings were coded as continuous to utilize response surface methods to find the best settings to reach a specified target. While the industrial example is trivial the characteristics of the project lend themselves to illustrate the complexity of real life applications.

AN INCREMENTAL FORWARD STAGEWISE REGRESSION ALGORITHM FOR DICHOTOMOUS RESPONSE VARIABLES. Adam Sima, Department of Biostatistics, Virginia Commonwealth University. The Incremental Forward Stagewise Regression (IFSR) procedure was developed by Hastie, et al. (2001) as a flexible estimation procedure for fitting penalized linear models. To generalize this procedure, the IFSR estimation method was extended for use with a dichotomous response variable. In particular, a simulation study was used compare both the accuracy in prediction and model fit to similar algorithms that simultaneously fit a model and estimate parameters. The results show that this method is comparable to some commonly used algorithms.

INTRODUCTION TO DISCRETE CHOICE MODELS. Bhaskara S. Ravi and N. Rao Chaganty, Department of Mathematics and Statistics, Old Dominion University. We often encounter with decisions that involve choosing between alternatives or choices such as “which phone to buy” or “which minute plan” to choose or “which brand of shampoo to buy” etc. Interestingly, these decisions not only depend on individual characteristics but heavily on alternatives available. Discrete choice models analyze such choice behavior and these are very popular in economics. This talk aims at introducing very famous McFadden’s conditional logit model and the importance of IIA (Independence of irrelevant attributes) assumption. Also, a review of current trends and challenges in this popular research area are presented.

Structural Biology, Biochemistry and Biophysics

MECHANISM OF ACTION OF UDP-GALACTOPYRANOSE MUTASE FROM TRYPANOSOMA CRUZI. Michelle Oppenheimer, Ana L. Valenciano, Jun Qi, & Pablo Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061. *Trypanosoma cruzi (T. cruzi)* is the causative agent of Chagas’ disease, which if untreated leads to chronic inflammation of the heart. UDP-galactopyranose

mutase (UGM) is a flavoenzyme that catalyzes the conversion of UDP-galactopyranose (UDP-Gal*p*) to UDP-galactofuranose (UDP-Gal*f*). UGM are important in parasitic pathogenesis and are absent in mammals, making UGM an attractive drug target. The chemical mechanism of UGM is not well understood. We have used steady-state kinetics, rapid-reaction kinetics, and trapping intermediates to better understand the mechanism of function of UGM. A k_{cat} value of 8.1 ± 0.3 and a K_M value of 43 ± 7 μM were determined for *T. cruzi* UGM (TcUGM) using steady-state kinetics. Spectral change indicative of a predicted flavin iminium ion were detected by rapid reaction kinetics analyses, consistant with a postulated flavin galactose adduct. The intermediate forms at a rate of 310 ± 40 s^{-1}, suggesting this is not the rate limiting step. We have trapped this intermediate and have been able to isolate the adduct by both the HPLC and identify it by mass spectrometry. Lastly, We sought to determine the redox partner for TcUGM. We show that TcUGM can be reduced by NAD(P)H and maintained the reduced state for several catalytic cycles. This activity is unique to eukaryotic UGMs as prokaryotic UGMs do not react with NAD(P)H. Supported by NIH grants GM094469 and AI082542 and the American Heart Association.

PROBING THE MECHANISM OF HIGH-FIDELITY DNA SYNTHESIS USING X-RAY CRYSTALLOGRAPHY. Eugene Wu, Department of Biology, University of Richmond, Richmond, VA. DNA polymerases replicate DNA with higher fidelity than would be expected from free energy differences between complementary and mismatched base pairs. One approach to studying replication fidelity is to determine X-ray crystal structures of reaction intermediates and polymerase complexes with mismatched base pairs. A crystal structure of a guanosine:thymidine triphosphate mismatch in the active site of DNA polymerase I from *Bacillus stearothermophilus* revealed a key intermediate conformation between the "open" and "closed" conformations. The previously unknown "ajar" conformation allows the template to interact with the incoming nucleoside triphosphate and position it relative to the polymerase active site. Complementary dNTPs advance past this conformation to a correctly aligned closed conformation for catalysis, while mismatches are misaligned, leading to substrate release. Other DNA polymerase I family enzymes share features important for the three-state sorting mechanism and are expected to use thia mechanism. One homologue with an altered active site sequence may show a preference for the ajar conformation. This homologue's crystal structure has been solved by multiwavelength anomalous dispersion and may lead to confirmation of the three-state or new insights into nucleotide selection by DNA polymerases.

INCORPORATION OF TAUTOMERISM INTO MOLECULAR MODELING; PROGRESS IN DEVELOPING PYRROLE-BASED ANTI-TUBULIN AGENTS. C. Da,[1] G. E. Kellogg,[1] N. Telang[2] & J. Gupton[2], [1]Virginia Commonwealth University, Richmond VA 23298-0540 [2]University of Richmond, Richmond VA 23173. Tautomerism is a commonly observed chemical phenomenon that involves readily available structural changes of the positions of protons and double bonds. However, alternative tautomeric forms are often ignored in many molecular modeling applications. In docking, inaccuracy in predicting binding affinities can be

related to the failure to consider all possible states including tautomeric states. We are working to incorporate tautomerism into modeling tools based around HINT (Hydropathic INTeractions). A preliminary version of the program has been developed to apply a simple, straightforward algorithm to identify and enumerate tautomeric forms. It is being used in an ongoing project of designing and developing pyrrole-based anti-tubulin agents, to identify and evaluate potential tautomers, and to build quantitative predictive models.

HIGH-THROUGHPUT ASSAY TO IDENTIFY INHIBITORS AGAINST UDP-GALACTOPYRANOSE MUTASE (UGM) FROM ASPERGILLUS FUMIGATUS. Jun Qi, Michelle Oppenheimer, & Pablo Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The flavoenzyme UDP-galactopyranose mutase (UGM) catalyzes the isomerization of UDP-galactopyranose to UDP-galactofuranose, the biosynthetic precursor of galactofuranose (Gal*f*). Gal*f* residues are essential components in the cell wall of pathogens and play vital roles for their virulence. Thus inhibitors of UGM that block the biosynthesis of Gal*f* could lead to novel therapeutics. To date, no eukaryotic UGM inhibitors and only a few prokaryotic UGM inhibitors have been reported. We present the development of a high-throughput fluorescence polarization (FP) assay to identify specific inhibitors of eukaryotic UGM from human pathogenic fungus *Aspergillus fumigatus*. Our FP binding assay demonstrates that specific binding to eukaryotic *Af*UGM were only obtained from UDP-TAMRA chromophores, and a UDP-TAMRA chromophore with K_d value of 2.6±0.2 μM was selected as the fluorescent probe in the high-throughput FP assay. The competitive binding assay indicates that this UDP-TAMRA chromophore shares the same binding site with UDP, a known UGM ligand that binds to the active site of UGM. The FP assay was evaluated and displayed excellent Z′ factor (0.79±0.01) and good tolerance to DSMO. Nine compounds were screened in this system, and one compound was identified as *Af*UGM ligand. This compound was further confirmed to inhibit the activity of *Af*UGM in *Af*UGM activity assay, indicating that the compounds identified by our FP high-throughput screening system are inhibitors of *Af*UGM. Supported by a NIH grant RO1-AI082542 (R. Tarleton PI).

HOMOLOGY MODELS OF C-C CHEMOKINE TYPE-5 RECEPTORS. ARE BOUND WATERS IMPORTANT IN BINDING SITES OF MEMBRANE BOUND RECEPTORS? Saheem A. Zaidi, Philip D. Mosier, Yan Zhang & Glen E. Kellogg, Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298. HIV-1 uses two plasma membrane receptors, CD4 and a co-receptor, to facilitate its entry into the target plasma membrane. Depending upon the type of virus, this co-receptor can either be C-C chemokine receptor type-5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4). Studies suggest that CCR5 plays a dominant role in early stages of infection. Many allosteric antagonists of CCR5 are known to inhibit fusion/entry process and subsequent infection of HIV-1. In the present study we explored an allosteric binding site of CCR5 and the possible role protein-bound water molecules may play in the interaction. A homology model of CCR5 was built using recently crystallized CXCR4 as a template, followed by virtual docking of maraviroc, a known allosteric antagonist. Mutagenesis studies

from the literature agreed with the docking pose obtained. Possible protein-bound water sites were generated and the antagonist was re-docked. According to our model there are at least two important protein-bound waters. One of the bound waters forms a hydrogen bond bridge between the ligand and the protein while the second bound water is displaced due to ionic-bridge formation between the ligand and the protein, and thus increasing the binding affinity due entropic gain resulting from disruption of water network in the unliganded receptor.

ANALYSIS OF POST-TRANSLATIONAL MODIFICATIONS OF SIKE FOLLOWING DOUBLE-STRANDED RNA STIMULATION. Charlotte F. Roberts, James D. Marion, R. Jason Call and Jessica K. Bell, Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. The innate immune system is the body's first line of defense against infectious agents. Essential to this response are cellular mechanisms that recognize, sequester and eradicate these invading organisms. Toll-like receptor 3 (TLR3), a pathogen recognition receptor, is stimulated by the viral genomic material double stranded RNA (dsRNA). TLR3 stimulation initiates a signaling cascade that leads to the production of type 1 interferon. Critical to this signaling pathway is a kinase complex, NAP1 (NAK associated protein 1)-TBK1 (TANK Binding Kinase 1)-IKKε (IκB kinase epsilon), which leads to the phosphorylation of IRF3 (interferon regulatory factor) and IRF7 and production of IFNβ. To control this activity, SIKE (Suppressor of IKKε) acts as a physiological inhibitor of IKKε and TBK1 activity through an undefined mechanism. The role of post-translation modifications to control SIKE function was examined. FLAG-tagged SIKE DNA was transiently transfected into HEK293 followed by dsRNA stimulation for 24 h. Cell lysates were harvested, SIKE immunoprecipitated using anti-FLAG antibody agarose beads. Immunoblot analysis showed serine phosphorylation state following dsRNA stimulation. Using in vitro kinetic assays, TBK1 mediated SIKE phosphorylation. These results suggest that upon pathway stimulation and TBK1 activation, TBK1 phosphorylates SIKE signaling SIKE's release from the kinase complex. Funding provided in part by the American Cancer Society.

SUPPRESSOR OF IKK-EPSILON IS A MIXED TYPE INHIBITOR OF THE TYPE I INTERFERON RESPONSE. James D. Marion, Charlotte F. Roberts, R. Jason Call, and Jessica K. Bell, Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. Kinases act in signaling pathways to propagate the cellular response by phosphorylation of specific targets that trigger downstream events that alter the cell's transcriptional program. To control kinase activity, the cell has developed an intricate series of protein interactions that are required for activation or mediate inhibition. In innate immunity, viral-derived ligands activate innate immune receptors to initiate an anti-response including production of type I interferons. Downstream of these receptors, a critical kinase complex, NAP1 (NAK associated protein 1)-TBK1 (TANK Binding Kinase 1)-IKKe (IkB kinase epsilon) phosphorylates of the transcription factor, IRF3 (interferon regulatory factor) and IRF7, which leads to the production of IFN-beta. To control this activity, SIKE (Suppressor of IKK-epsilon) acts as a physiological inhibitor the kinase activity through an undefined mechanism. Our goal is to define

the inhibitory mechanism of SIKE in the kinase reaction. Using an in vitro kinase assay, the Michaelis constant for IRF3 phosphorylation was determined (5 micromolar). Upon addition of increasing concentrations of SIKE, a decrease in Vmax for IRF3 phosphorylation was observed with minimal change in Km. Further analysis showed that SIKE functioned as a mixed type inhibitor with an approximate IC50 of 450 nM. The effect of SIKE phosphorylation on inhibitory function is currently under study. Funding provided in part by the American Cancer Society.

STRUCTURE BASED PREDICTIVE MODELS FOR PROTEIN POST-TRANSLATIONAL MODIFICATIONS. M. Zhang[1] , V. A. Yakovlev[2], R. B. Mikkelsen[2] & G. E. Kellogg[1], [1]Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, VA 23298 [2]Department of Radiation Oncology, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298. Nitric oxide (NO) has long been identified as a diffusible signaling molecule, but only until recent years had gained prominence as an inducer of redox-based protein post-translational modifications (PTMs). NO-dependent PTM is achieved, in a large part, through cysteine S-nitrosylation, the covalent addition of the NO moiety to a reactive cysteine thiol (other modes of action also exist, such as tyrosine nitration). Over the last decade, the number of reported substrates for S-nitrosylation has grown exponentially, and are shown to participate in a wide range of biological processes, however, the precise mechanism of S-nitrosylation is still elusive, the only certainty is that targeting of S-nitrosylation between proteins, and especially between Cys residues within substrate proteins reflects the interactions of a number of determinants of specificity. In an attempt to untangle these determinants, we have developed computational methods to build models of protein post-translational modifications based on 3D structural features, which have been successfully applied to predict tyrosine nitration. In the current study, we are developing models that we hope can accurately predict Cys S-nitrosylation. Preliminary analysis with 821 cysteines has proven to be promising, some interesting interactions between structural features have been identified with a logistic regression model, and a much simpler but more accurate model was built with a novel ant-colony algorithm.